

Synchronous Growth of *Escherichia coli* after Treatment with Fluorophenylalanine

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Treatment of exponentially growing *Escherichia coli* cells with the amino acid analogue, *p*-fluorophenylalanine (FPA), resulted in a decrease in the amount of deoxyribonucleic acid (DNA) present per cell. A minimum DNA content per cell was reached approximately 80 min after addition of FPA to the culture. After this time the DNA per cell rose to the level of the untreated cell. When an FPA-treated culture was resuspended in fresh medium containing no FPA 80 min after addition of the analogue, the increases in cell number and DNA became synchronous. Ribonucleic acid was synthesized immediately and continuously throughout the cell cycle, except during periods of DNA synthesis and cell division.

When auxotrophs of *Escherichia coli* are deprived of required amino acids, deoxyribonucleic acid (DNA) replication ceases at a particular point on the chromosome (7). Reinitiation of replication will not take place unless the required amino acids are supplied. Thymine starvation also blocks chromosome replication at the growing point and at the unique region reached during amino acid starvation (5). This region is specific and inheritable and has been proposed to be the chromosomal origin (8).

Carpenter and Binkley (2) have shown that treatment of *E. coli* with 10^{-2} M *p*-fluorophenylalanine (FPA) blocks replication at the same point as that reached in the absence of amino acids. Replication will be reinitiated when FPA is removed from the medium. The possibility was investigated that FPA-treated cells might reinitiate replication simultaneously from the origin and consequently divide synchronously when suspended in FPA-free medium.

MATERIALS AND METHODS

Growth of *E. coli* B and determination of cell number were described in the preceding paper (1).

Nucleic acids were determined in the following manner. Cold trichloroacetic acid was added to 15-ml portions of growing culture to give a final concentration of 10%. Two drops of a solution of bovine serum albumin containing 30 μ g/ml were added to each sample as coprecipitant. Samples were kept at 4°C overnight and centrifuged at $12,100 \times g$ for 10 min. The pellet was washed three times with 5-ml portions of 10% trichloroacetic acid. The nucleic acid was extracted into 2 ml of 0.5 N perchloric acid by heating for 40 min at 70°C.

A 1.5-ml amount of extract was used for the DNA

assays. DNA was determined according to the procedure of Ceriotti (3) with the modification of Keck (4). A 1-ml amount of extract was used for the determination of ribonucleic acid (RNA). The orcinol method was used to determine this component (10).

RESULTS

Figure 1 shows the increase in cell mass, the increase in cell number, and the DNA content per cell after treatment of an exponentially growing culture of *E. coli* with 10^{-3} M FPA. After addition of FPA, cell mass increased at a linear rate. Approximately 2 hr later, cell mass began to increase at a slow exponential rate with a generation time of 120 min. Cell division was blocked during the latter part of the linear phase of growth. With the start of the slow exponential phase, cell number increased at a rate slightly greater than that of mass increase. These observations were reported previously by our laboratory (9). At 50 min after addition of FPA, the amount of DNA per cell began to decrease. This measurement became minimal approximately 30 min later. Beyond this time, the DNA content per cell rose until the level of the untreated cell was attained. At this stage cells were growing at a slow exponential rate.

Since work of Carpenter and Binkley (2) suggested that high concentrations of FPA block replication of the chromosome at the origin, it was suspected that the time of lowest DNA content per cell represented a time at which replication of the chromosomes of most cells was inhibited and at the origin. If a certain population of cells were phased at the origin of the chromosome by FPA, then removal of the inhibitor

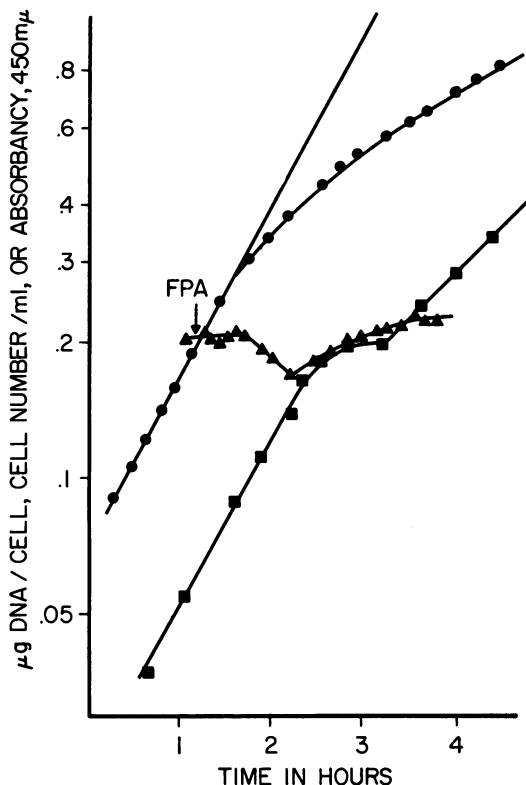


FIG. 1. Increase in cell number and DNA content per cell of a culture treated with FPA. Symbols: \blacksquare , cell number per milliliter, $\times 10^{-9}$; \blacktriangle , micrograms of DNA per cell, $\times 10^{10}$; \bullet , absorbance, 450 nm.

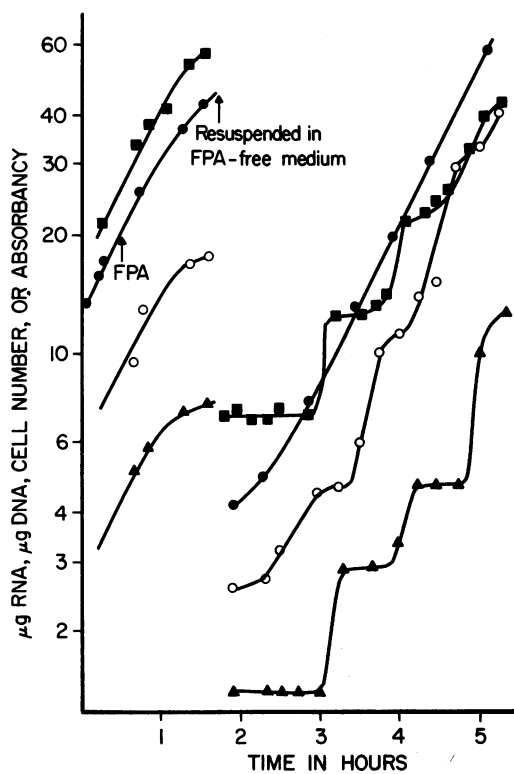


FIG. 2. Preparation of a synchronized culture from cells treated with FPA. Symbols: \blacktriangle , micrograms of DNA per 15 ml of culture; \blacksquare , cell number per milliliter $\times 10^{-7}$; \circ , micrograms of RNA per ml of culture; \bullet , absorbance at 450 nm, $\times 10^8$.

should allow these cells to initiate replication simultaneously.

To test the hypothesis, 100 ml of an exponentially growing culture of *E. coli* was treated with FPA and, 80 min later, it filtered onto a 0.45- μ m pore size filter (Millipore Corp., Bedford, Mass.). The cells on the filter were suspended immediately in 800 ml of warm medium lacking FPA. Increases in cell number, DNA, and RNA were followed throughout the experiment (Fig. 2). After suspension in FPA-free medium, cell number and DNA increased synchronously, that is, stepwise. RNA was synthesized immediately after resuspension of the cells. However, the rate of synthesis was slowed during the period of DNA synthesis and cell division. This decreased rate was always observed during cell division in the first two cycles. Synchronous growth could be maintained through three doubling times.

DISCUSSION

The fact that synchronous growth can be acquired after treatment of *E. coli* with FPA

lends further support to the hypothesis that FPA blocks replication at the origin of the chromosome. If a certain portion of the cell population could reinitiate replication from the same point on the chromosome at the same time, it is expected that these cells would synthesize certain cellular components synchronously and also undergo cell division synchronously.

Studies of Lark and Lark (6) with the DNA inhibitor, phenethylalcohol, suggested that RNA synthesis is required for initiation of replication. In our system, RNA is doubled before DNA synthesis begins. This result is therefore in agreement with Lark's conclusions.

This method of producing synchronized growth may offer certain advantages. (i) The wild-type of *E. coli* can be used, that is, a mutant is not required. (ii) A period of only 80 min of treatment with FPA is necessary. Time is then not a limiting factor, and (iii) once the analogue is removed, the 45-min generation time is resumed. (iv) Large quantities of cells can be handled.

An alternative to filtration is to add phenylal-

anine to the culture. Results obtained by this procedure are not as satisfactory as for the method described.

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